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A modified trap design for sampling subterranean habitats for central Texas *Eurycea* salamanders

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Abstract

In this paper, we describe modifications to a sampling technique for surface, stream-dwelling salamanders for use in subterranean settings. Leaf litter bags are an effective and commonly used trap for salamanders, and their construction purposefully allows animals to move freely in and out of the trap. However, this presents a problem in subterranean deployment because retrieving the trap over long vertical distances, such as well sampling, allows time and space for the animals to escape. To overcome this challenge, we enclosed a leaf litter bag in a suspended net system contained by a lanyard to sample a 3-meter deep well. Our trap modifications resulted in the live capture of adult and immature federally threatened Salado Salamanders (*Eurycea chisholmensis*) from the well in addition to aquatic invertebrates. This represents a novel trapping technique within a habitat system for which stygofauna sampling options are limited.

Keywords

Active trap, Amphibian sampling, Karst Biology, Leaf litter trap, Salado Salamander, Stygofauna, Threatened Species

Introduction

Knowledge of stygofauna and their ecology is inevitably less available than that of epigean fauna because the subterranean habitats stygofauna occupy are less accessible for sampling (Hahn 2002; Larned 2012; Hose et al. 2017). Conducting manual biological surveys (i.e., hand collection) may be a preferred way to collect

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stygofauna, but this requires a point of access to the subterranean environment in the form of geologic features such as caves or sinkholes. The occurrence of these geologic features is often rare (Larned 2012), especially features large enough to accommodate human passage.

Subterranean sampling and trapping techniques have been developed to survey areas inaccessible to humans. Commonly employed methods for sampling stygofauna have been restricted to the hyporheic zone using water well or bore sampling, as well as the deployment of traps (Larned 2012). Hyporheic zone sampling often involves the extraction of interstitial water through methods such as hyporheic pumping, which allows researchers to collect organisms residing in the water-sediment interface. For deeper groundwater environments, bottle traps or funnel traps are commonly employed. These are typically deployed in wells or at cave entrances to capture fauna inhabiting these otherwise inaccessible zones. While these methods primarily target invertebrates, such as crustaceans and other small aquatic organisms (Benedict 1896; Fenolio et al. 2015, 2017; Külköylüoğlu et al. 2017a, 2017b, 2017c), targeted sampling strategies are essential for vertebrates when they do occur in these environments.

Despite extensive research on invertebrate sampling, methods for effectively sampling subterranean vertebrates remain less established. Although the use of bottle and funnel traps to sample for stream-dwelling (i.e., epigean) salamanders is also well documented (e.g., Richter 1995; Mushet et al. 1997; Fronzuto and Verrell 2000; Wilson and Dorcas 2003; Nowakowski and Maerz 2009), their effectiveness for subterranean vertebrates is limited and not well documented (McDermid et al. 2015). Despite their utility, bottle traps and funnel traps have drawbacks. Because these traps restrain captured animals, they must be checked frequently to prevent mortality, a critical consideration when researching endangered and threatened species (Wilson and Gibbons 2010).

A popular technique for sampling surface (i.e., epigean) stream-dwelling salamanders is the use of leaf litter bags (Pauley and Little 1998; Jung et al. 2000; Waldron et al. 2003; Edwards et al. 2016). Leaf litter bags attract and concentrate, rather than trap, fauna by providing favorable structure objects, and animals are able to freely move in and out of the bag (Willson and Gibbons). This makes leaf litter bags a favorable technique when deploying traps for long periods because there is less risk of accidental death from target fauna becoming truly trapped and not being able to leave the trap (Waldron et al. 2003). This is especially important for listed endangered or threatened species, where the health and well-being of the animals takes precedence.

In surface waters, researchers check leaf litter bags by quickly sliding a tray or sieve under the bag to catch any target fauna that may fall through the mesh netting (Willson and Gibbons 2010). However, this presents a problem in subterranean deployment because traps often need to be retrieved vertically through the water column (or air), allowing ample time for fauna to escape. Here, we present a technique to remedy this problem to allow the effective use of leaf litter bags in subterranean settings.

The study species

The target of this sampling methodology was Salado Salamanders (*Eurycea chisholmensis*), a fully aquatic groundwater obligate salamander. Salado Salamanders are the northernmost species within a radiation of central Texas *Eurycea* salamanders and are endemic to groundwater habitats north of the San Gabriel River and south of Salado Creek in Williamson and Bell counties, Texas. (US Fish and Wildlife Service 2014; Devitt et al. 2019). Because of anthropogenic pressures and concerns of reduced water quantity and quality within the Edwards/Trinity aquifer system that contains this radiation of distinct species, *E. chisholmensis* along with several other species in this radiation have been listed under the Federal Endangered Species Act or are being prepared for future proposed listings (e.g., U.S. Fish and Wildlife Service 2013, 2014).

Methods

Study area

Cobbs Spring is ephemeral and discharges from the northern segment of the Edwards Aquifer in the Berry Creek watershed and is located on private property within Williamson County, Texas, USA. The soil and associated geology near the spring consists of silty and clayey slope alluvium underlain by Edwards limestone. Pecan (*Carya illinoinensis*) is the dominant overstory vegetation surrounding the spring. Within the spring run, the Edwards limestone is weathered entirely exposing the topmost Comanche bedrock.

Early settlers of this property installed an approximately 3 m deep hand-dug well (Fig. 1) located about 100 m downstream of the spring outlet and 50 m west of the main channel of the spring run. We observe this well gaining and losing water in correspondence with discharge volume in the spring itself, and thus we assume it is fed by the same source of groundwater. We visited Cobbs Spring monthly as part of an ongoing capture-mark-recapture study of *Eurycea* salamanders at select Williamson County sites (Cambrian 2020, 2021). Salamanders are previously known to occur within the well (U.S. Fish and Wildlife Service 2014), and we opportunistically observed salamanders in the well over the course of population monitoring.

Trap design

We modify a commonly used and documented trapping technique for stream-dwelling salamanders (Jung et al. 2000; Waldron et al. 2003; Edwards et al. 2016; Peirson et al. 2016) for use in subterranean aquatic applications. Although the factors influencing stygofauna occurrence are not well studied, some research indicates that the most important factors are habitat structure and the supply of organic matter (Korbel and Hose 2015; Ercoli et al. 2019). The method we describe herein adds a potential foraging environment to a sparse environment. This may attract salamanders either for its

Figure 1. Hand cut limestone well under a pecan tree. The well near Cobbs Spring is a relatively shallow well made of limestone blocks that were cut by hand. Situated under a pecan tree, the well is uncovered, allowing leaves and organic matter to naturally fall inside.

use as habitat structure or by first attracting salamander prey species with food in the form of organic leaf matter, which in turn attract salamanders.

Our goal when designing this trap method was to create a leaf litter bag that could be lowered into a well within a mesh bag (Figs 2, 3), which when lowered to the bottom of the well would lay flat allowing salamanders access to the leaf litter trap (Fig. 4), but upon extraction enclose around the leaf litter trap (Fig. 5), ensuring capture of all animals harbored within. Leaf litter bags are typically fashioned from polypropylene mesh netting designed for landscaping; leaf litter is collected and contained within the poly netting. Although the netting is typically used to exclude deer or birds, salamanders are able to enter and leave the 'trap' freely because the 2.5 cm wide squares that make the netting are large enough for several salamander species to pass through. We constructed a leaf litter bag approximately 30 cm × 30 cm × 45 cm in size using "Vigoro Polypropylene Deer Block Netting, UV Treated" (Model # NMVDB07100 Store SKU #295769 purchased from Home Depot) which has 1 inch (2.54 cm) apertures, and cable ties. The leaf litter used was collected at the site and composed mostly of Pecan, which also naturally occurs inside the well from the surrounding canopy. We used native leaf litter collected at the site because a change to the quality or quantity of litter entering caves has the potential to disrupt the structure and function of cave communities (Hillis et al. 2008). Additionally, Pecan leaves are larger than the 2.5 cm aperture of the deer block netting allowing them to be contained satisfactorily. The bottom of the trap was made from a "Leslie's Pro grade 18 inch Leaf Rake" (Sku:

Figure 2. Leaf litter bag and modified pool net. A leaf litter bag is held above a modified pool bag, demonstrating the trap used for collecting and examining aquatic salamanders.

82627 LPM #: 82627; manufacturer location - HQ - Phoenix Arizona) brand pool skimmer. This device features a fine mesh whose apertures are smaller than we would expect any *Eurycea* salamander, at any life stage, to be able to escape through. The pool skimmer was deconstructed and only the mesh bag was used to fashion the trap. A wire coat hanger was modified into a circle and mounted onto the rim of the pool skimmer bag with cable ties. In order to lower the trap into the well, 30lb test monofilament line was tied to four areas on the rim of the wire coat hanger circle. With the leaf litter bag inside the pool skimmer bag, the trap was lowered to the bottom of the well, adjusting the position so that the pool skimmer bag laid flat along the bottom of the well.

The trap was checked four hours after first deployment (Fig. 6), it was then redeployed and rechecked three times, each one month apart coinciding with our capture-

Figure 3. Lowering the Leaf Litter Trap into the Well. The image shows the trap being lowered into the well using the lanyard. The leaf litter bag, contained within the pool net, is carefully guided down to the bottom of the well, positioning it to lay flat and become accessible to salamanders. This setup ensures a smooth descent and accurate placement of the trap in the well.

Figure 4. Leaf litter bag with no lanyard tension. Illustrating how the leaf litter bag is fully accessible to salamanders when the pool net lies flat against the bottom of the well, with no tension on the lanyard. This design ensures that the leaf litter bag provides unobstructed access to the trap contents, allowing salamanders to enter and leave freely.

Figure 5. Leaf litter bag with lanyard tension. Demonstrating the trap in its closed position with tension applied to the lanyard, causing the pool net to enclose the leaf litter bag. This configuration securely captures the salamanders and prevents their escape, ensuring all animals within are retained during extraction.

Figure 6. First captured *Eurycea chisholmensis* with this trap. The first two federally listed *E. chisholmensis* salamanders captured using the modified leaf litter trap. One is an adult, and the other is a juvenile, showcasing the trap's effectiveness in capturing different life stages. Photos of these salamanders were taken to be able to identify them as recaptured in successive surveys using the Wild ID software.

mark-recapture study. We retrieved the trap by hoisting it out of the well by the 30lb test monofilament line. Each time the trap was checked, water from the nearby spring was collected and then poured over the leaf litter bag as it was gently shaken to allow any invertebrates and salamanders to fall out of the leaf litter bag and into the pool skimmer bag. While processing the invertebrates and salamanders that ended up in the pool skimmer bag, we placed the leaf litter bag over a sieve (Adcock et al. 2022) in case any more invertebrates or salamanders remained in the leaf litter bag after the initial washing and shaking. Salamanders were categorized as juvenile if their total length was approximately <25 mm based on a visual estimate (Pierce et al. 2010) and also checked for gravidity (i.e., the presence of oocytes within the translucent venter). Salamanders were then photographed against a 5 mm background to be later measured for total length and snout-trunk length (STL) and to identify individuals (Bolger et al. 2012; Bendik et al. 2013). Salamanders were then replaced into the pool skimmer bag along with the leaf litter trap and returned to the well, as the trap system was redeployed. Representative specimens of each captured invertebrate were also vouchered, and excess captures were released back into the well, following the same protocol as with the salamanders. Recaptured salamanders were identified from dorsal photos based on their unique chromatophore patterns using the free open-source photo recognition software 'Wild ID', a tool designed to identify individuals based on unique patterns from photos instead of using a physical tag on the animal (Bolger et al. 2012). Salamanders were measured from photos using the free open-source software 'Image J', using the 5 mm grid background as a reference.

Results

A total of 18 salamanders were captured, representing 12 unique individuals (determined through capture mark recapture methods) (Table 1). Multiple salamanders were captured during every sampling event, and we never collected an injured or dead salamander (Table 2). We additionally captured four species of invertebrates representing three Classes (Insecta, Clitellata, and Gastropoda), as well as Rio Grande Leopard Frog tadpoles (*Lithobates berlandieri*).

Discussion

Stygofauna are understudied, and many species within this group are of conservation concern due to their cryptic nature (Hahn 2002; Simões et al. 2013). These species are often granted protected status, such as threatened or endangered, under the Endangered Species Act, necessitating research and data collection (Doremus and Pagel 2008). Consequently, there is a critical need to develop and refine survey techniques to provide reliable sampling options for practitioners working to conserve and manage these vulnerable species.

Although this water well is shallow and open to the surface, we expect the methods we describe above to be applicable to a variety of sampling requirements and unique situations. The successful capture of salamanders within this well, given its proximity

Table 1. Salado Salamander (*Eurycea chisholmensis*) measurement and gravidity data.

Date	Salamander Unique ID	Total Length (mm)	STL (mm)	Gravid
31 Oct 2019	C0432	24.1	14.6	No
	C0433	47.1	25.9	No
20 Nov 2019	C0434	32.4	18.3	No
	C0435	33.7	18.6	No
	C0436	43.2	23.0	No
19 Dec 2019	C0432	24.9	15.8	No
	C0454	43.6	25.7	No
	C0435	33.6	19.3	No
27 Jan 2020	C0435	34.7	19.0	No
	C0470	40.6	22.5	No
	C0471	30.3	17.7	No
24 Feb 2020	C0493	32.6	19.2	No
	C0494	31.5	20.0	No
	C0495	34.0	21.0	No
	C0470	41.2	23.9	No
	C0471	31.6	20.4	No
	C0496	42.2	24.5	No
	C0432	28.4	17.7	No

Table 2. Salado Salamander (*Eurycea chisholmensis*) capture results using the modified leaf litter trap.

Date	Deployment Time	Total Adult Captures	Total Juvenile Captures	Recaptured Individuals
31 Oct 2019	4 hours	1	1	–
20 Nov 2019	20 days	3	0	0
19 Dec 2019	29 days	2	1	2
27 Jan 2020	39 days	3	0	1
24 Feb 2020	28 days	7	0	3

to the known occupied spring, demonstrates the use of subterranean habitat by this species within the karst subterranean habitat that underlies the uplands.

The recapture of 6 unique individual salamanders over five sampling events without mortalities demonstrates that salamanders can enter and leave the trap safely. This trap was designed specifically for this well, but it could potentially be used as a sampling technique for other wells, caves, and hard-to-reach places in occupied springs. This methodology may also have utility to inventory invertebrates that utilize leaf litter, and in federally mandated occurrence surveys for this group of salamanders (USFWS 2021). With some modifications, we expect that this trap can be deployed in various deep-water settings, caves, solution cavities, and wells of different sizes and depths. Additionally, the leaf litter bag could be replaced by other artificial structures meant to attract fauna, such as mop heads, which are commonly used to sample both groundwater salamanders and invertebrates (e.g., Gibson et al. 2008; Devitt and Nissen 2018). There are additional species within the genus *Eurycea* that are federally listed and continue to be understudied, especially in subterranean habitats. This sampling method holds potential for effective use in studying the majority of this genus within the Edwards/Trinity aquifer system, given the shared life history strategies observed among most central Texas *Eurycea* species.

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